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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 10/616,659 | 07/09/2003 | Costas D. Maranas | P06367US03 | 9959 |

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| EXAMINER | |
|---|---|
| SKOWRONEK, KARLHEINZ R | |
| **ART UNIT** | **PAPER NUMBER** |
| 1631 | |

| SHORTENED STATUTORY PERIOD OF RESPONSE | MAIL DATE | DELIVERY MODE |
|---|---|---|
| 3 MONTHS | 02/16/2007 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/616,659 | MARANAS ET AL. | |
| | Examiner | Art Unit | |
| | Karlheinz R. Skowronek | 1631 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *19 December 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) *6, 9 and 15-17* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-14 and 18-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

### *Claim Status*

Claims 1-20 are pending.

Claims 6, 9, and 15-17 are withdrawn.

Claims 1-5, 7-8, 10-14, and 18-20 are being examined.

### *Response to Arguments*

Applicants' arguments to the objections/rejections stated in the previous office action have been fully considered and are persuasive in part. Rejections not reiterated hereby withdrawn. The following rejections constitute the complete set presently being applied to the instant application.

### *Priority*

As stated in the office action dated 19 September 2006, Application No. 60/395,763, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for claims 1-14 and 18-20. Application No. 60/417,511 and Application No. 60/444,933 provide support for the claims. Thus benefit of priority is only granted from the date 9 September 2002.

### *Claim Rejections - 35 USC § 112*

The following rejection is reiterated from the previous office action dated 19 September 2006.

Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the formation of a bilevel linear programming optimization problem, does not reasonably provide enablement for the formation of any

optimization problem. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claim 1 is dawn to a method using a model of an organism's metabolic pathways to determine potential genes which could be modified by deletion or addition in order to obtain a desired product or effect via the steps: selecting a bioengineering objective; selecting at least a cellular objective; and forming an optimization problem.

*Breadth:*

The breadth of the claim is so wide as to encompass any model of any organism, multiple cellular objectives, any bioengineering objective, and forming and solving any optimization problem.

*Nature:*

The nature of the invention relies on a model of a metabolic network and the algorithm capable of describing/forming any optimization problem, the calculations performed and selection/decisions required to predict the candidate genes in the metabolic network.

*State of the Art:*

The state of the art at the time of invention had been applying the statements of mathematics, such as Linear Programming optimization problems (cf. Schilling et al, Biotechnology and Bioengineering, 71(4):286-306, 2001) and algebraic optimization problems, to the analysis and modeling of metabolic networks to predict the "phenotypes" of alterations to the reaction steps that make up a metabolic pathway for a

particular organism. The optimization problems have been analyzed with various computational environments, such as MATLAB (cf. Klamt et al., Bioinformatics, 19(2):261-269, 2003). However, the formation of an optimization problem and concomitant mathematical statement and solution of the problem presents a complex, computational hurdle that remains to be overcome. Methods, such as network division or metabolite grouping have be practiced to reduce the computational complexity of the optimization problems, but these methods introduce other issues, like the overlaps between many metabolite biosynthetic pathways (Papin et al., 5/2003, Trends in Biochemical Sciences, 28(5):250-258, p.256, "computational obstacles").

*Lack of guidance:*

The specification provides guidance for the formation of a bilevel optimization problem. However, the specification does not provide guidance for forming any optimization problem. The specification does not specifically define "optimization problem". Giving the term "optimization problem" its broadest meaning, it is interpreted to mean any method that results in attaining a biological objective and a cellular objective. The specification also provides no examples of the formation of other optimization problems.

*Level of ordinary skill:*

One of ordinary skill in the art would be able to make the invention that practices a limited number of known optimization problems. However the breadth of claim is such that optimization problems that have not been conceived or novel are also included. One of ordinary skill in the art would not be able to make the invention without undue

experimentation which is required to conceive and develop a novel optimization problem.

Thus undue experimentation would be required for one of ordinary skill in the art to make or use the invention within the full scope of claims.

***Response to Arguments***

Applicant argues that the specification describes the use of linear programming as one means to solve an optimization. However as stated in the rejection above, the specification does not enable any method of optimization. The claims are broadly drawn to any optimization method, whereas, the specification is directed to linear programming optimization methods only. Thus given the breadth of the claims, optimization methods not yet conceived and that lack description in the specification are also included.

Applicant argues that in case where computational resources and time are not limited or where the biological network is sufficiently small there exists no computational hurdle. The Papin et al reference addresses this point specifically, that even for small reaction networks an algorithm that can solve the problem in polynomial time does not exist (p. 256, col 2, para. 1, lines 1-12). It therefore presents an undue burden on one of ordinary skill in the art to determine which networks can be disregarded, consolidated or otherwise subject to reduction in order to produce a computationally tractable problem.

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The following rejection is reiterated from the previous office action dated 19 September 2006.

1. Claims 1-5, 7-8, 10-14, and 18-20 rejected under 35 U.S.C. 103(a) as being unpatentable over Burgard et al. (Biotechnology and Bioengineering. 2001 74:364-375), in view of Yang et al. (metabolic engineering, 1999, 1:26-34) and further in view of Voit (Biotechnology and Bioengineering, 1992, 40: 572-582).

To the extent of the elected species, claims 1-5, 7-8, 10-14, and 18-20 are drawn a method of identifying gene candidates for deletion and addition by forming and solving a bilevel optimization problem that involves a bioengineering objective, e.g. lactate overproduction and a cellular objective, e.g. maximizing growth in an organism.

Burgard et al. teach a method of identifying gene candidates for deletion and addition by forming and solving an optimization problem that involves a bioengineering objective and a cellular objective ("Mathematical modeling of gene additions/deletions", p367-369).

Burgard et al. do not teach the generation of a bilevel optimization problem; also the reference does not teach lactate overproduction as a "bioengineering objective".

Yang et al. teach lactate overproduction in E. coli by the deletion of the phosphotransacetylase gene, PTA, and the acetate kinase gene, ackA. Yang et al also demonstrate the underproduction and overproduction of chemicals, specifically acetate and lactate, relative to the wild type strain. One of ordinary skill in the art will also recognize lactate as a carbon source used in by E. coli during anaerobic fermentation.

Voit teaches the application of bilevel programming optimization ("multilevel programming", p.572), techniques as S-system, to bioengineering of organism strains ("design of pathways", p. 579) by solving the optimization problem to identify candidates for deletion and addition ("to optimize a yield", p.573 and p.576).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use the bilevel optimization of Voit in the method of Burgard et al. to develop the strain of E. coli that overproduce lactate as taught by Yang et al. One would have been motivated to combine the teachings of Burgard et al., Yang et al and Voit to result in the instantly claimed invention, by Yang et al. because the organism, E. coli, is " extensively used in industry as a host for recombinant protein production (Yang et al, p. 26-27)" and by Voit because bacteria could be used to produce unnaturally high amounts of oxychemicals that can be used as alternative fuels (p. 579)" and recombinant protein production and would have reasonably expected success in view of the teachings of Burgard et al and Voit. Accordingly the invention as a whole is *prima facie* obvious.

With respect to the limitations of claim 2, drawn to modifying an organism with a candidate, is also taught by Yang et al. in which the *pta* gene of E. coli was knocked out to generate a *pta* deficient organism (see table 2a, p. 27, Yang et al.).

With respect to the limitation of claim 7, drawn to a candidate deletion and a binary value specifying if a reaction is active or inactive, is also taught by Burgard et al. Burgard et al. teach the use of a binary value to specify if a reaction is active or inactive, "the binary parameter, $a_{jk}$, is defined to describe which enzymes are coded for by which genes: $a_{jk}$ = 0 if gene k has no direct effect on reaction j; 1 if gene k codes for an enzyme catalyzing reaction j ("binary parameter", p367-368, Burgard et al.). This reads on the limitation of claim, the assignment of a binary value to a reaction flux. The limitation of deletions is taught in, "In this study we explore what is the smallest gene set capable of maximizing biomass production on glucose substrate (uptake 10mmol) and what is the maximum number of gene deletions from this gene set that still maintains a specified level of biomass production (p.369)".

The above statement also teaches the limitations of claim 13 drawn to the evaluation of performance limits ("smallest gene set"), the limitations of claim 20 and 14, drawn to an objective corresponding to maximizing growth rate, and the limitations of claim 5, drawn to growth ("maximizing biomass production"). The title of Burgard et al. also reads on the limitations of claim 13, performance limits.

With respect to the limitations of claim 11, drawn to a chemical uptake constraint, is also taught by Burgard et al., "quantifies the network's uptake (if negative) or secretion (if positive) of metabolite i. (p. 366)" and "stoichiometric coefficient of

metabolite i (p.366)". Metabolites are known in the art to chemical in nature and stoichiometric coefficient is a constraint.

With respect to the limitation of claim 12, drawn to quantifying the cellular objective as an aggregate flux, is also taught by Burgard et al. as "maximized the biomass production flux, $v_{max\ biomass}$. The solution yields the maximum theoretical level of biomass production ($v_{max\ biomass}$ = 1.25g biomass/gDW·h) achievable by the metabolic network within the stoichiometric constraints (p. 369)".

With respect to the limitation of claim 10, drawn to at least one stoichiometric, is also taught by Burgard et al. in "These upper bounds are set by maximizing the given flux $n_j$ subject to the stoichiometric constraints (p. 369)".

With respect to the limitations of claim 19 are intrinsic to the teaching of Burgard et al., "These problems are solved using CPLEX 6.6 accessed via the commercial software package GAMS. Problems with up to 3700 binary variables were solved on an IBM RS6000-270 workstation (p. 369)".

***Response to Arguments***

Applicant's arguments filed 19 December 2006 have been fully considered but they are not persuasive. Applicant argues none of the references, Burgard et al, Voit et al or Yang et al teach the limitation of forming an optimization problem that couples the at least one cellular objective with at least one bioengineering objective. However Burgard et al clearly teach the limitation in the abstract, lines 4-14:

> *"Both the gene addition problem of optimally selecting which foreign genes to recombine into E. coli, as well as the gene deletion problem of removing a given number of existing ones, are formulated as mixed-integer optimization*

> *problems using binary 0–1 variables. The developed modeling and optimization framework is tested by investigating the effect of gene deletions on biomass production and addressing the maximum theoretical production of the 20 amino acids for aerobic growth on glucose and acetate substrates."*

Thus, Burgard et al do teach the required limitation. Since Burgard et al teach the limitation, it would be obvious and one would have been motivated as described above to combine the references.

Regarding applicant's second argument, that the references do not teach claim 19 limitation of quantifying the cellular objective. This is not persuasive because Burgard teach in table III quantified predictions of the solution to the optimization problem. The problems as discussed above are formulated in the form of a computer program (cf. Burgard, p. 369).

Applicant's third argument is made on the grounds that there exists no proper motivation or obviousness to combine the three references. This is not found persuasive. It would be obvious to one of ordinary skill to make a prediction of a pathway and then test the prediction in an organism of choice. All scientific endeavors are based on a similar principle, i.e. iterations of hypothesis generation and hypothesis testing. One of ordinary skill would have found a sufficient amount suggestion and motivation from the Voit, Burgard et al and Yang et al references to combine. First, all three references are directed to the formulation of computational problems to describe the dynamics of the metabolic pathway for an organism of interest using mathematical modeling. For example, Voit teaches that given the potential of designing and genetically engineering pathways it is necessary to develop mathematical methods for

analyzing, controlling, and optimizing integrated biochemical systems. Indicating the obviousness for doing so (cf. Voit, p. 572). Burgard et al similarly teach that as the prediction capabilities of metabolic models continue to improve, the effect of multiple gene deletions on network (biochemical) robustness and organism survivability can be studied with increasing confidence (cf. Burgard et al, p. 364). Yang et al teach that metabolic engineering is a useful tool for providing precise perturbations of physiological studies whose effects can be examined using metabolic flux analysis (cf. Yang et al, p. 27). Second, the Yang et al reference teaches the application of mathematical model predictions to actual modifications practiced in an organism. For example, Yang et al apply the predictions of metabolic models to lactate production in E. coli. The Burgard et al and Voit references suggest the combination of mathematical biochemical pathway models with an actual practice of the predictions in an organism (cf. Voit, p. 573 and Burgard et al, p. 374). Finally, all three references each provide one of ordinary skill with the motivation to combine. Burgard et al motivate one to combine metabolic modeling to produce production pathways with enhanced production. Voit motivates one to combine through the teach that as reliable kinetic information regarding biochemical pathways and networks of medical and industrial interest grow, the information can be used to optimize the pathways and networks to achieve goals of interest, such as stimulating a bacterium to produce unnaturally high amounts of oxychemicals. Yang et al motivates one combine using the specific example of reduction of acetate production in *E. coli*; teaching that the reduction of acetate accumulation has been shown to enhance recombinant protein production. Yang et al further teach that E. coli is extensively used

as a host in industry for recombinant protein production because of the ease of manipulation, wealth of genetic information, fast growth rate, standardized cultivation techniques and inexpensive growth media. Thus one would have had the motivation to combine the references used in the instant rejection. Based on the success of Yang et al and the previous successes referred to by Burgard et al and Voit in the application of metabolic prediction to enhance the yield of a product of interest, one would have had a reasonable expectation of success.

***Conclusion***

No claims allowable.

**THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karlheinz R. Skowronek whose telephone number is

(571) 272-9047. The examiner can normally be reached on Mon-Fri 8:00am-5:00pm (EST).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on (571) 272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Karlheinz R. Skowronek/

MICHAEL BORIN, PH.D
PRIMARY EXAMINER